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ABSTRACT

The effects of elevated CO_2 partial pressure (pCO_2) on otolith calcification and on the coupling between the somatic and otolith growth were investigated in juvenile gilthead seabream *Sparus aurata*. Six-month old individuals were raised during seven weeks under four pCO_2 conditions set according to projected future ocean acidification scenarios. Body and otolith biometric parameters were measured throughout the experiment along with the otolith biomineralization monitored using a radiotracer technique based on ^{45}Ca incorporation. Seabream exhibited somatic growth resilience to all treatments. In contrast, increased growth rate and shape complexity of otoliths were observed with a pH_T drop from 8.1 to 7.5. Hypercalcification was observed under lowered pH, with a rate of calcium incorporation increasing by up to 18% between pH_T 8.1 and pH_T 7.7. This work highlighted an uncoupling of otolith and body growth of juvenile seabream within 40 d at pH_T 7.9 projected to be reached by the end of the century. As the otolith is an essential tool used in reconstructing fish life history, this work suggests that information resulting from otolith studies should be interpreted with caution with respect to the potential impacts that ocean acidification projected modifications could have on otolith biomineralization.

Keywords: climate change; ocean acidification; otolith calcification; somatic-otolith growth allometry; temperate coastal fish

1. INTRODUCTION

On 9th May 2013, the concentration of carbon dioxide (CO_2) in the atmosphere reached the symbolic threshold of 400 ppm in Mauna Loa, Hawaii (IPCC, 2013), a level never reached at this reference site. The increase of atmospheric CO_2 from a preindustrial value of 280 µatm is the result of fossil fuel combustion, cement production and land use change (Ciais et al., 2013).

IPCC projections suggest further increase in the coming decades with concentrations reaching 490 ppm in 2050 and more than 1370 ppm by 2100 (IPCC, 2013). The ocean is a major carbon sink, absorbing about 25% of anthropogenic CO_2 emissions thus limiting the greenhouse gas effects on climate (Le Quéré et al., 2013). The increase in pCO_2 in the ocean has already led to a pH decline of 0.1 unit since the industrial revolution and to major shifts in the ocean carbonate chemistry, *i.e.* increased concentrations of dissolved inorganic carbon and bicarbonate ions, decreased carbonate concentration, altogether leading to 'ocean acidification' (Caldeira and Wickett, 2005). The pH of the surface ocean is expected to decline by 0.06 to 0.32 units by the end of century (Ciais et al., 2013), resulting in an unprecedented change of seawater chemistry equilibrium since the last 800,000 years (Zeebe and Ridgwell, 2012).

For a decade, a growing body of experimental studies has examined the response of marine organisms to decreased pH across multiple taxa. Impacts on early life stages have been of particular concern in fish (Baumann et al., 2012) because it has been hypothesized that animal embryos and larvae may not be as resilient to physiological stress as juveniles and adults (Pörtner, 2008), and because recruitment cohorts lay the foundation for population success and connectivity (Planes et al., 2009). Nevertheless juveniles of coastal fish may be more exposed to high CO_2 levels than earlier life stages and deserves particular attention. Indeed, unlike in the open ocean, seawater pCO_2 is known to vary considerably in coastal waters depending on land-driven eutrophication, which adds to the uptake of atmospheric CO_2, locally amplifying ocean acidification (Cai et al., 2011; Guinotte and Fabry, 2008). Juvenile fish settling in coastal areas during their high metabolic and fast growing phase could be severely challenged by hypercapnic conditions.

Based on the sparse current knowledge, adult physiological performance allows fish to cope with extracellular acidosis caused by increasing pCO_2 (*e.g.* Melzner et al., 2009b). But in early-life stages of multiple taxa including fish, increasing pCO_2 was shown to affect calcification of shells

and skeletons due to a drop in the carbonate availability (*e.g.* Gattuso et al., 1998; Riebesell et al., 2000). Munday et al. (2011a) observed no effects on spiny damselfish otolith calcification at 850 μatm, while Munday et al. (2011b) and Checkley et al. (2009) highlighted an otolith hypercalcification in white sea bass larvae exposed at 993 and 2558 μatm pCO_2 and in clownfish larvae at 1721 μatm pCO_2, respectively. In cases of calcification modulation, otolith morphology can be affected, which may have negative repercussions on the behaviour and acoustic function in fish, decreasing their survival probabilities (Bignami et al., 2013a; Popper et al., 2005). It has also been recently reported that otolith increment growth can be uncoupled from somatic growth in fish larva raised under high pCO_2 conditions, then having potential implications for the study of fish populations (Bignami et al. 2013b). Understanding the way ocean acidification can affect the process of otolith formation is decidedly important in many perspectives among which is seems crucial to delineate how the environment can affect its growth and influence its coupling with the structural skeleton growth. If otoliths indeed ensure the fish's biological hearing and balance functions, they are also an essential tool used in fisheries biology to reconstruct individual life history in terms of age and somatic growth relationship (age-length keys) and attended habitats (Campana and Neilson, 1985; Campana, 2005).

This paper aims at evaluating the impacts of ocean acidification on the calcification rate of fish otoliths and on the understudied coupling between otolith and somatic growth in juveniles. These questions were investigated experimentally using a nuclear tracking approach, following the ^{45}Ca incorporation in otolith for 7 weeks of exposure to 4 realistic pCO_2 levels projected for the near-future. The gilthead seabream *Sparus aurata* was chosen due to its ecology and high economic value. Temperate and widely distributed over the North-Eastern Atlantic Ocean and the Mediterranean Sea, this coastal species is subjected to recreational and professional fishing and is increasingly aquacultured (FAO, 2014). In the Mediterranean Sea, it is the first marine fish species cultured with more than 140,000 tonnes produced in 2010 in 17 countries for a worth *ca.*

US$ 785 million (GFCM, 2013).

2. MATERIALS AND METHODS

2.1 Organisms, radiotracer and experimental procedures

Six-month old juveniles of seabream *Sparus aurata* have been purchased at the "Cannes Aquaculture" fish farm of Monaco and were placed for three weeks in an open-circuit 500 l tank in the IAEA-EL premises for acclimation.

Then, 200 fish juveniles of *ca.* 50 mm were randomly assigned in four 20 l circular tanks (one tank per treatment) filled with filtered (0.45 µm) and UV-sterilized Mediterranean seawater (38 p.s.u.) pumped from 30 m depth in the Bay of Monaco. In each experimental tank (closed system), seawater was constantly aerated. The light/dark cycle was 12h/12h. Fish were fed daily *ad libitum* with pellets. After feeding, not ingested food has been removed and 80% of the volume was renewed daily with sterilized and filtered seawater. Temperature was maintained at 21°C and controlled in each bath to within ± 0.5°C using temperature controllers connected to 300 W submersible heaters. Seawater pH (pH_T on the total scale, Dickson et al., 2007) was adjusted to the desired level from ambient pH_T of 8.1 (corresponding to 475 µatm of pCO_2) to low pH_T of 7.9, 7.7, and 7.5 (700, 1200 and 2000 µatm, respectively), as derived from various models on trajectories of carbon emissions to the near-future (IPCC, 2013). The pH was controlled in each bottle to within ± 0.05 pH unit using a pH-stat system (IKS, Karlsbad). The experimental containers were continuously bubbled with CO_2-free air and discrete amounts of pure CO_2 were added by the pH-stat system. pH and alkalinity were measured and set according to Martin et al. (2011) using the R package seacarb (Proye and Gattuso, 2003).

Seawater in each bottle was spiked with ^{45}Ca (10 kBq l^{-1}). Radiotracers were purchased from Radioisotope Centre Polatum, Poland, ^{45}Ca [as $^{45}CaCl_2$; $T_{½}$ = 163 d]. Stock solutions were prepared in H_2O to obtain radioactivities that allowed the use of spikes of only a few microliters

(typically 5-10 µl). The radiotracer spikes were renewed at each water change and were checked (i.e. counted in 1 ml of seawater) before and after each water renewal in order to maintain radiotracer concentrations.

Fish were maintained for 40 d at the four pH treatments and continuously exposed to dissolved ^{45}Ca in seawater. Five fishes were collected every 3 or 7 d in each tank, anesthetized with clove oil prior to decerebration and then measured in length and weight to the nearest 0.1 mm and 1 mg, respectively. Left and right otoliths (sagittae) were extracted and photographed using a Leica DFC420 camera mounted on a stereomicroscope (Leica LZ12). Otolith surface area (OSA, mm^2), maximum Feret diameter (OF, mm) and roundness (OR) were calculated using ImageJ software and weight measurement was made to the nearest 0.1 mg. ^{45}Ca content was then determined. Paired radiolabelled sagittal otoliths from the same individual were pooled and dissolved adding 300 µL of hydrochloric acid (HCl, 37%) at 80°C. After evaporation, the residues were dissolved in 1 mL of distilled water. Biological and seawater samples were counted after adding 10 mL of scintillation liquid, Ultima GoldTM XR (Perkin Elmer). Emissions were measured with a liquid scintillation analyzer (Tri-Carb, Packarb 1600 TR or Perkin Elmer 2900 TR) calibrated with an appropriate standard for each counting that was used. Counting times were adapted to obtain relative propagated errors less than 5% (from 10 min to 24 h). Corrections for the physical half-life time and background noise were done in order to determine the ^{45}Ca activities at the sampling time (Bq). The uptake of Ca in the otolith was then expressed as the amount of Ca incorporated (Q_{Ca}, in µmol Ca g^{-1} of otolith dry wt) and following the equation (Martin et al., 2011):

$$Q_{Ca} = [(A_{cut} / A_{sw}) \times C_{sw}] \times 10^3$$

where A_{cut} is the total ^{45}Ca activity in each otolith (in Bq), A_{sw} is the time-integrated activity (in Bq g^{-1}) in seawater during the time of exposure and C_{sw} is the total Ca concentration in Mediterranean seawater (0.0114 mmol g^{-1}).

151

152 *2.2 Data Analyses*

153 Due to compromises with experiment cost, radioprotection rules and waste management
154 possibilities with respect to the use of radioisotope ^{45}Ca, only one 20-l tank has been dedicated to
155 each pH conditions. Therefore, fish sampled along the experiment course at regular sampling
156 time (n = 5 (and 10) per sampling time (at 40 d) per condition) have been considered as
157 pseudoreplicates (Hurlbert, 1984). Accordingly, the effect of pH on measured physiological or
158 morphological responses of fish has been tested through multiple linear regression considering
159 the pH as a continuous covariate (Havenhand et al., 2011). Linear regressions were used to test
160 the effects of pH (continuous covariate), on relationships between otolith biometrics (*i.e.* otolith
161 surface area (OSA), weight (OW), maximum diameter (Feret's diameter, OF), roundness (OR))
162 and fish total length (FTL) or Time, including a FTL (or Time) x pH interactions. When FTL (or
163 Time) x pH interaction was not significant, a simpler model without interaction (*i.e.* Time (or
164 FTL) + pH) has been computed (Crawley, 2005).
165 To evaluate the effect of the pH treatment on the asymmetry of paired-otoliths (regarding left-
166 right differences in OSA, OF and OR), log-transformed data were injected in a Brown-Forsythe
167 robust Levene test of homogeneity of variances based on deviation from the median.
168 The amount of Ca incorporated in otoliths were standardized to the otolith weight and expressed
169 according to the Time of experiment. Ca incorporation data (mmol g^{-1} otolith dry weight) have
170 been fitted by multiple linear regression with Time x pH as continuous covariates.
171 Results are expressed as mean ± s.d. Significance was considered at $p<0.005$. All statistical
172 treatments have been done using R (R Core Team, 2013).

173

174 3. RESULTS

175 *3.1 Culture conditions*

During the experiment, the fish biomass in each tank did not exceed 5 $g.l^{-1}$, *i.e.* far below the EU recommendations for fish welfare (CEE n°2092/91) which limit the biomass to 25 $g.l^{-1}$ for fish aquaculture in marine environment. Along the 40 d of ^{45}Ca exposure, pH_T was maintained at a mean (± s.d.) of 7.50 ± 0.06, 7.69 ± 0.03, 7.89 ± 0.05 and 8.04 ± 0.05 (Table 1), corresponding to pCO_2 of *ca.* 2000, 1200, 700, and 475 µatm, respectively. Mean temperature was 21.0°C ± 0.7. Mean A_T of renewed seawater was 2.595 ± 0.003 $mmol.kg^{-1}$. It changed by a maximum of 0.2 $mmol\ kg^{-1}$ between two seawater renewals. C_T increased from 2410 to 2690 $\mu mol\ kg^{-1}$ whereas the CO_3^{2-} concentration decreased from 240 to 80 $\mu mol\ kg^{-1}$ with decreasing pH. In all conditions, the saturation state with respect to aragonite was higher than 1.

3.2 Somatic versus otolith growth

Fish somatic growth in total length and weight was significant over the 40 days period of the experiment (p-value < 0.001, Table 2), slightly inflecting from day 30 (Figure 1, A,B). As a mean, fish grew by 0.36 $mm.d^{-1}$ and 0.043 $g.d^{-1}$, a growth rate close to the 0.0534 $g.d^{-1}$ value measured in juveniles seabream (1.24 ± 0.02 g) reared in closed system tank at a density of 1.5 $g.l^{-1}$ (Kalogeropoulos et al., 1992). Similarly Kim et al. (2012) reported a mean weight growth rate of 0.0364 $g.d^{-1}$ for young juveniles of red seabream reared from 1.46 g for 42 days, arguing for adequate rearing conditions in our experiment allowing a normal growth and development of the juveniles (Figure 2). The pH treatments had no impact on those body growth parameters and intercepts did not differ between treatments meaning the initial group of juveniles was homogeneous (p-value > 0.05). In contrast, the allometric relationship between otolith surface area and fish total length (Figure 3.A), though still linear, was altered by the decreasing pH (p-value < 0.001, Table 2) with a lower slope value observed at pH_T = 8.1 compared to pH_T = 7.9 and 7.7, and even more compared to pH_T = 7.5 (Figure 3.A). The otolith's Feret diameter and fish total length relationship tended also to be modified in the two lowest pH conditions though

not significantly (Figure 3.B, Table 2). The otolith roundness was not related to fish total length except at pH_T 7.5 where the linear relationship was low but significant (Figure 3.C, p-value < 0.0046) indicating a faster evolution of the otolith toward a less round shape along with body growth at the lowest pH than at more elevated ones. Finally, the otolith weight was linearly correlated to the otolith surface area during all the experiment and whatever the pH treatment (Table 2), indicating that the density of the otolith was not affected by pCO_2.

3.3 Asymmetry

Levels of both surface area and roundness asymmetry between left and right sagittal otoliths (Figure 4) of juvenile seabream were not affected by pH treatments over the 40 days (Brown-Forsythe robust Levene Test based on deviation from the median; $p > 0.05$). Disequilibrium of the symmetry in the otolith surface area toward a greater OSA of the right otolith was observed during the first half of the experiment whatever the pH (Figure 4.A.), but disappeared in animals sampled during the last 10 days of experiment. No asymmetry of the otolith roundness was observed (Figure 4.B.).

3.4 Calcification

The calcification of otolith was followed through ^{45}Ca accumulation throughout the 40 days of experiment (Figure 5). The incorporation of Ca in otolith of juvenile seabreams followed a linear equation whatever the pH (Table 3), which is consistent with the linear growth of fish over the experiment. Based on the slope calculated for each pH, we could estimate that for the fish reared at pH_T 8.1 rates of Ca incorporation were 13, 17 and 18% lower than rates performed at pH_T 7.9, 7.7 and 7.5, respectively.

4. DISCUSSION

226 Assessing whether elevated pCO_2 and lowered pH impact marine fauna and flora and to what
227 extent it can affect individual, population and ecosystem function is a major concern (Fabry et
228 al., 2008; Hilmi et al., 2013; Melzner et al., 2009a). Recent studies have reported that
229 hypercapnia can challenge fish physiology (e.g. Melzner et al., 2009b; Michaelidis et al., 2007),
230 affect the behaviour (Simpson et al., 2011) and growth (Baumann et al., 2012) and impact
231 calcified structures biomineralized from the early-life stages (Checkley et al 2009, Ries et al.
232 2009). Our study shows that responses can be contrasted at the sub-individual integration level.
233

234 ***4.1 Effect of elevated pCO_2 on the coupling of fish somatic and otolith growth***

235 Juvenile seabream *Sparus aurata* reared under 4 pCO_2 treatments during 40 d exhibited no
236 variation in somatic growth. As documented, physiological performance of fish could be
237 expected to allow them to cope with elevated pCO_2 (Melzner et al., 2009b) without significantly
238 affecting the animal's growth (Hurst et al., 2012; Ishimatsu et al., 2008). Michaelidis et al.
239 (2007) demonstrated that long-term hypercapnia may cause a shift from aerobic to anaerobic
240 metabolism in seabream *Sparus aurata*, but only at dramatically elevated pCO_2 (~5000 μatm).
241 This suggests that this species is able to cope with realistic increasing pCO_2, according to IPCC
242 scenarios, without extra-energy demand and might thus maintain its juvenile somatic growth rate
243 as observed in this study.
244 In contrast, a modulation of the otolith biomineralization was observed, with an increase of the
245 calcification rate observed from 700 μatm after 40 days of exposure. This means that juvenile
246 seabream might be subjected to the hypercalcification of their otoliths by the end of the century
247 with respect to IPCC projected CO_2 levels. Only Bignami et al. (2013b) reported such a short-
248 term effect of increased pCO_2 under the threshold of 800 μatm, and this was at the larval stage of
249 cobia *Rachycentron canadum*. Here, the higher growth rate of the otolith surface area under

250 rising pCO_2 was directly linked with the increase of the net calcification rate calculated on the
251 basis of ^{45}Ca incorporation. The linearity of calcium incorporation with time was not disrupted
252 by pCO_2 treatments, but it did provide evidence of a critical difference in the mineralisation
253 slopes. The hypermineralization of calcareous structures in organisms maintained in decreasing
254 pH and in less favourable chemical condition for $CaCO_3$ precipitation (lower carbonate
255 saturation state, Ω_{arg}) has been already observed in species of various taxonomic groups
256 characterized by efficient capacities to maintain blood/hemolymph homeostasis, such as
257 crustaceans (Ries et al., 2009), cephalopods (Dorey et al., 2012; Gutowska et al., 2010) and
258 fishes (Bignami et al., 2013b; Checkley et al., 2009). Indeed, the active uptake of bicarbonate
259 ions in internal medium to compensate blood acidosis is expected to contribute at increasing Ω_{arg}
260 in the endolymph fluid surrounding the aragonitic otolith (Borelli et al., 2003). The precipitation
261 of HCO_3^- with Ca^{2+} in endolymph leads in turn to active proton extrusion to avoid impeding the
262 calcification process (Allemand et al., 2007). Nonetheless, the cellular mechanisms underlying
263 the hypercalcification of otoliths in this context of ocean acidification remains poorly understood
264 (Munday et al., 2011b) and require further insights.
265 The observed uncoupling of fish body and otolith growth may have implications in fisheries
266 studies. Indeed, back-calculation of fish size from the otolith size is a common and quite highly
267 effective tool used in fisheries biology (Campana and Neilson, 1985; Mosegaard et al., 1988;
268 Payan et al., 1997). It can be used to determine fish-prey size spectra from otoliths collected in
269 the stomach contents of predators (Blanco et al., 2001; Markaida and Sosa-Nishizaki, 2003; Ross
270 et al., 2005). It is also commonly used to infer fish-size at age, based on otolith growth-
271 increments reading (Secor and Dean, 1989). However to be reliable, body and otolith growth
272 have to be uniquely and confidently linked throughout the developmental stage or time period of
273 interest. A body of studies has shown how complex this relationship can be (reviewed in Wilson
274 et al., 2009), being potentially impacted by environmental parameters fluctuations such as

275 temperature (Mosegaard et al., 1988; Takasuka et al., 2008). To date, "growth-effect" and "age-
276 effect" were the two major explanations of the uncoupling between somatic and otolith growth
277 (Wilson et al., 2009). This work presents the perspective that the distortion of the fish-otolith
278 allometric relationship can also originate from the modulation of the otolith growth induced by
279 variations of environmental parameters to which body growth is (more) resilient. Ocean
280 acidification, as a compound of the global change, appears here to alter this relationship.
281
282 ***4.2 Effect of increased pCO_2 on otolith shape***

283 Ocean acidification has also been reported to affect other otolith metrics that were monitored
284 during this experiment. As otolith weight increased in the same way as the otolith surface area,
285 no effect of pCO_2 increase up to 2000 μatm was observed on seabream otolith density. These
286 results are consistent with Bignami et al. (2013b) who did not observe any density modification
287 of the otolith in cobia larvae until 800 μatm. Nevertheless, they reported an increase of 6%
288 density when they experimentally increased conditions to 2100 μatm, suggesting an alteration of
289 the otolith's mass-size relationship under extreme hypercapnia conditions. Seabream otolith
290 shape seems to be also sensitive to pH conditions. Sagitta of juveniles reared at 475 μatm where
291 indeed significantly shapeless compared to those mineralized at 2000 μatm. As no asymmetry
292 between left and right otoliths was recorded, no sign of "anomalous" calcification appeared,
293 being in agreement with Maneja et al. (2013) who did not observe a deviation from symmetry in
294 otoliths of Atlantic cod larvae raised at 1800 μatm and even at 4200 μatm. Then, the here-
295 observed lower otolith roundness with high pCO_2 seems to provide evidence that in response to
296 hypercapnia, the otolith not only grows, but also develops more rapidly, acquiring at a higher
297 rate its species-specific ornaments (Campana and Casselman, 1993; Popper et al., 2005).

298 All these otolith descriptors are important in the otolithic apparatus functioning, as the main
299 organ of the ear in the senses of balance, directed motion detection and hearing (Lychakov and
300 Rebane, 2000; Popper et al., 2005). The otolith features indeed determine the compromise made
301 between these three senses. In bottom-dwelling species such as *Sparus aurata*, otoliths tend to be
302 bigger relative to the body size than in pelagic species (Popper et al., 2005), improving balance
303 and hearing senses while impairing motion detection. Hearing in seabream has been shown to be
304 a developed sense that is critical for the welfare of juveniles (Filiciotto et al., 2013). Thus, as
305 reported in another Perciform and bottom-dwelling species (Bignami et al., 2013a), ocean
306 acidification, in increasing the size of the otolith relative to the size of the body, may improve
307 the hearing acuity of *S. aurata*. As highlighted by Bignami et al. (2013a) improving auditory
308 capacity could also be advantageous or deleterious depending on the sound spectra newly
309 accessible to the fish. As Filiciotto et al. (2013) reported, wild offshore sound at low pressure
310 levels are more beneficial to *S. aurata* with individuals being less stressed and faster growing
311 than individuals exposed to constant and high sound pressure levels in aquaculture tanks. In
312 increasing hearing acuity, ocean acidification could thus induce a threshold shift of sound
313 pressure tolerance (Smith et al., 2004) or displace the optimum window of frequency, making
314 the baseline sound of the environment more disturbing and stressful (Simpson et al. 2011, Caiger
315 et al., 2012; Filiciotto et al., 2013). As many other ecologically and economically important
316 species fished in the wild and cultured in offshore farms, the consequences of ocean acidification
317 on growth and survival performances related to balance and auditory capacities need to be
318 considered and evaluated.
319
320 ***4.3 Potential repercussions of elevated pCO_2 on the otolith as a recorder of the seawater***
321 ***chemistry***
322 Considerations have also to be raised in regard to the use by fisheries scientists of the chemical

composition of the otolith as a biological tracking tool (Campana, 2005; Miller et al., 2006). Here, calcium incorporation into the otolith was modulated by seawater pH. This questions the stability of the elemental:Ca ratio under environmental hypercapnia. During the biomineralization of the otolith, chemical elements such as metals and metalloids are supposed to substitute for calcium (Campana, 1999) or, at least for some transition metals, complex with the organic matrix of the otolith via its constitutive metal-binding proteins (Miller et al., 2006). The changes of pH and seawater chemistry caused by increased CO_2 can modify the speciation of metals and therefore their bioavailability to organisms (Millero et al., 2009). The physiological response of fish to hypercapnia might in turn stimulate processes to compensate for acidosis based on the key role of ion transporters such as the Na^+/H^+ exchangers (Hu et al., 2013) that are hypothesized to be a major accumulation pathway for some cationic elements (Webb and Wood, 2000). In this context, ocean acidification may interfere with trace element uptake and body concentrations (Lacoue-Labarthe et al., 2009) and therefore could affect microchemical signature recorded in fish otolith. Two previous studies observed that ocean acidification has no effect on alkaline metals Ba-, Mg-, Sr-Ca ratio in the otolith of larval and juvenile fish (Hurst et al., 2012; Munday et al., 2011a). In contrast, in statoliths of squid larvae, the activity of the transition metal ^{65}Zn has been shown to increase with lowering pH (Lacoue-Labarthe et al., 2011). Future research thus seems still needed to investigate the possibility that ocean acidification could impact the incorporation of other trace elements used to track movements of marine organisms (Arkhipkin et al., 2004; Campana et al., 2000), depending on their chemical properties, molecular-binding affinities and incorporation pathway into the otolith.

5. Conclusions

In conclusion, this study demonstrates that, even under projected near-future pCO_2 levels,

juvenile seabream exhibited an increase of their otolith calcification and development rates while their body growth rate was not affected. Highlighting an uncoupling of otolith and body growth rates which appeared within 40 d at a pCO_2 of 700 µatm projected to be reached by the end of the century, this study shows that juvenile seabream could be more resilient to the ongoing ocean acidification in terms of somatic growth than in terms of structural calcification. As the otolith is an essential tool used in reconstructing fish life history in terms of age, somatic growth and attended habitats, this work suggests that information resulting from otolith studies should be interpreted with caution with respect to the potential impacts that ocean acidification projected modifications could have on otolith biomineralization.

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550 **TABLES**

551

552 **Table 1.** *Sparus aurata*. Carbonate chemistry and pH (mean ± sd) in the four pH levels applied
553 to reared juvenile of seabream for 40 d (1 tank per treatment). Partial pressure of CO_2 (pCO_2; µatm),
554 dissolved inorganic carbon (DIC; µmol.kg^{-1}), CO_3^{2-} concentration (µmol.kg^{-1}) and saturation state of seawater with
555 respect to aragonite (Ω_{arg}) are calculated from pH_T, temperature (21.0°C), salinity (38) and the total alkalinity
556 varying with time between two water renewals according the following equation: TA (µmol. kg^{-1}) = 2599 + 196 x
557 Time (day). Data are means ± s.d. of measurements taken every 15 min from Day 0 to Day 40; N = 24,724.

558

Treatment	pH_T	pCO_2	DIC	CO_3^{2-}	Ω_{arg}
pH 8.1	8.04 ± 0.05	477 ± 78	2412 ± 341	238 ± 22	3.65 ± 0.34
pH 7.9	7.89 ± 0.05	726 ± 101	2506 ± 247	178 ± 15	2.73 ± 0.23
pH 7.7	7.69 ± 0.03	1198 ± 105	2603 ± 153	121 ± 8	1.85 ± 0.13
pH 7.5	7.50 ± 0.06	1978 ± 202	2685 ± 244	80 ± 13	1.22 ± 0.19

559

560 **Table 2.** *Sparus aurata*. Parameters of the multiple linear regressions between fish total length
561 (FTL, mm) and Time (days), fish total weight (FTW, g) and Time (days) otolith surface area
562 (OSA, mm^2) and FTL, otolith weight (OW) and OSA, otolith Feret maximum diameter (OF,
563 mm) and FTL, and pH considered as a continuous co-variable, measured over the 40 days period
564 of experiment in 4 tanks maintained at pH 7.5, 7.7, 7.9 and 8.1. Significance of *p*-values ^{NS} >
565 0.05; * < 0.05; ** < 0.01; *** <0.001.

Model	Parameter	Variable p-value	Model F-value	Model p-value
FTL *vs.* Time	Time	< 0.001***	117.1	< 0.001***
	pH	0.654 ^{NS}		
FTW *vs.* Time	Time	< 0.001***	85.8	< 0.001***
	pH	0.372		
OSA *vs.* FTL	FTL	0.002**	246.9	< 0.001***
	pH	0.046*		
	FTL x pH	0.021*		
OW *vs.* OSA	OSA	< 0.001***	737.3	< 0.001***
	pH	0.772 ^{NS}		
OF *vs.* FTL	FTL	0.002**	280.2	< 0.001***
	pH	0.636 ^{NS}		

566 Model degree of freedom: 3 and 186

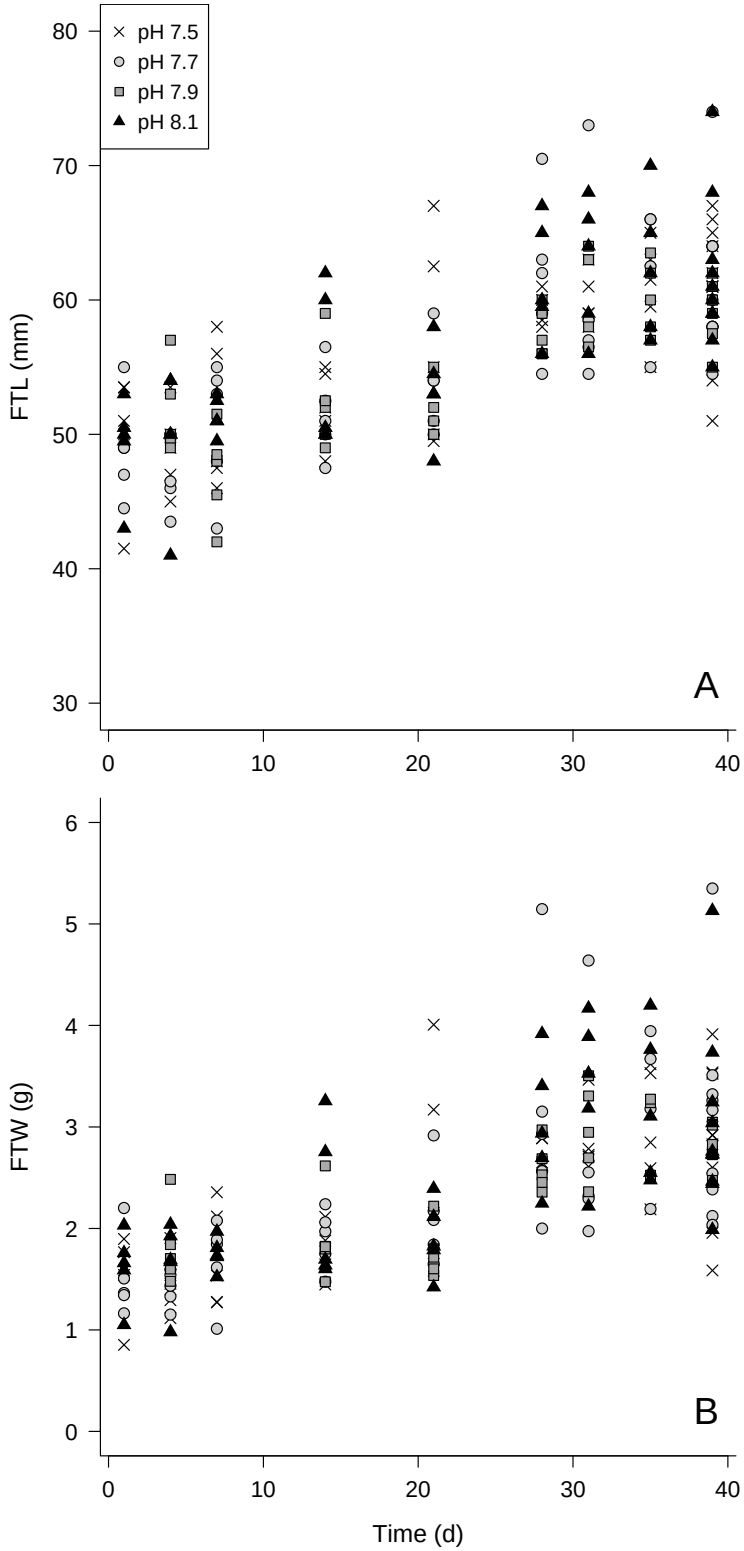
567 **Table 3.** *Sparus aurata.* Daily rate of calcium incorporation ($mmol.g_{otolith\ dry\ weight}^{-1}.d^{-1}$; mean ±

568 sd) in otolith and parameters of the multiple linear regressions with pH as continuous factor.

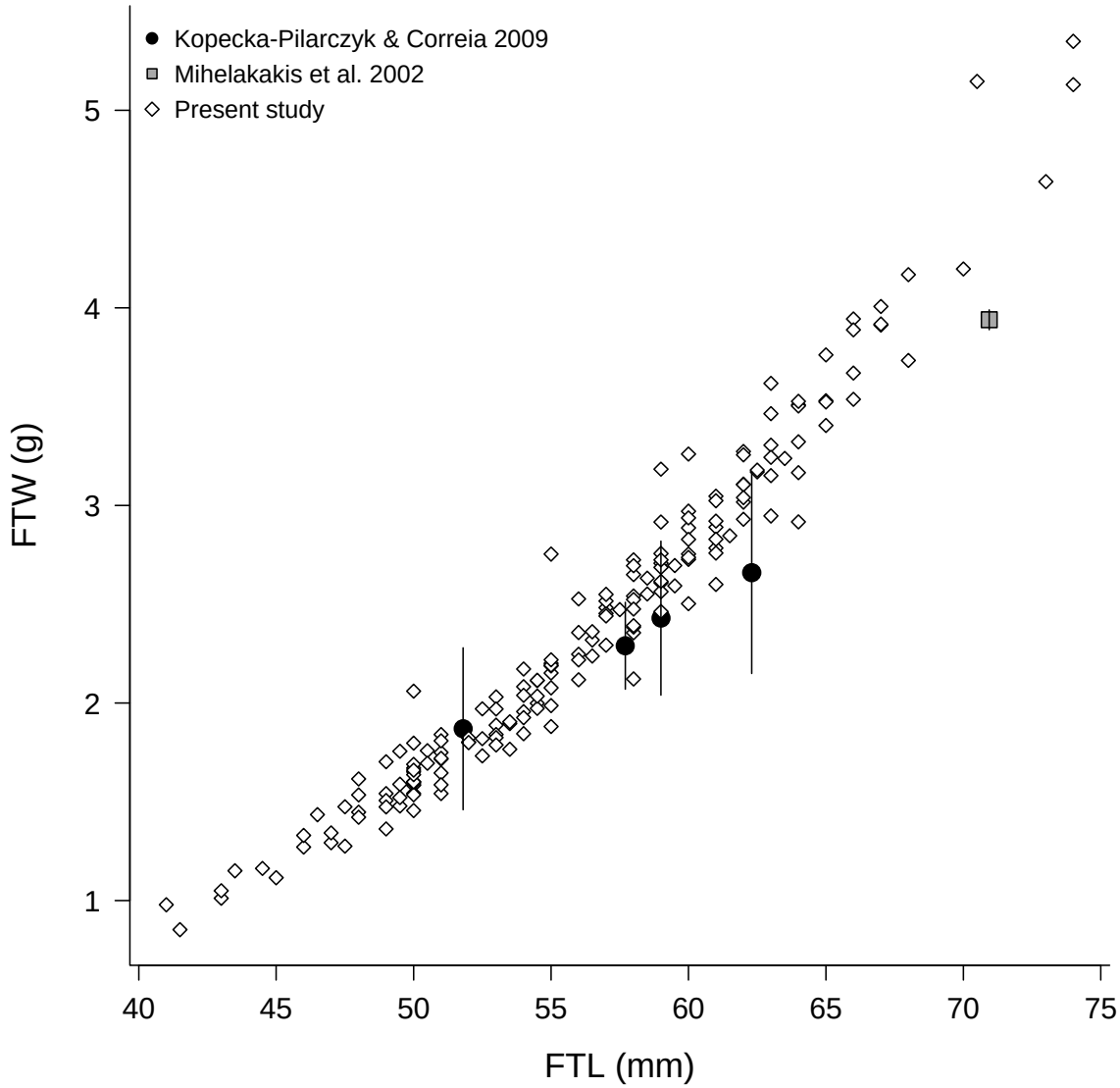
pH_T	N	Ca incorp. rate	R^2	Multiple linear regression		
				Parameters	Par. p-value	Model
pH 8.1	49	0.052 ± 0.001	0.962	Time	< 0.001	F test: 1753
pH 7.9	48	0.060 ± 0.002	0.969	pH	0.369	P < 0.001
pH 7.7	49	0.062 ± 0.002	0.974	Time x pH	< 0.001	
pH 7.5	50	0.062 ± 0.001	0.965			

569 R^2: determination coefficient; Model degree of freedom : 3 and 187 df.

570 **FIGURES**



571
572 **Figure 1**. *Sparus aurata*. (**A**) Fish total length (mm) versus time (days of experiment) in the
573 different pH conditions. (**B)** Fish total weight (g) versus time (days of experiment) in the
574 different pH conditions.



575
576 **Figure 2**. *Sparus aurata*. Fish total weight (g) versus fish total length (mm) recorded
577 individually in this study (all 4 pH conditions merged) and means (±sd for weight) reported by
578 Mihelakakis et al. (2002) and Kopecka-Pilarczyk and Correia (2009).

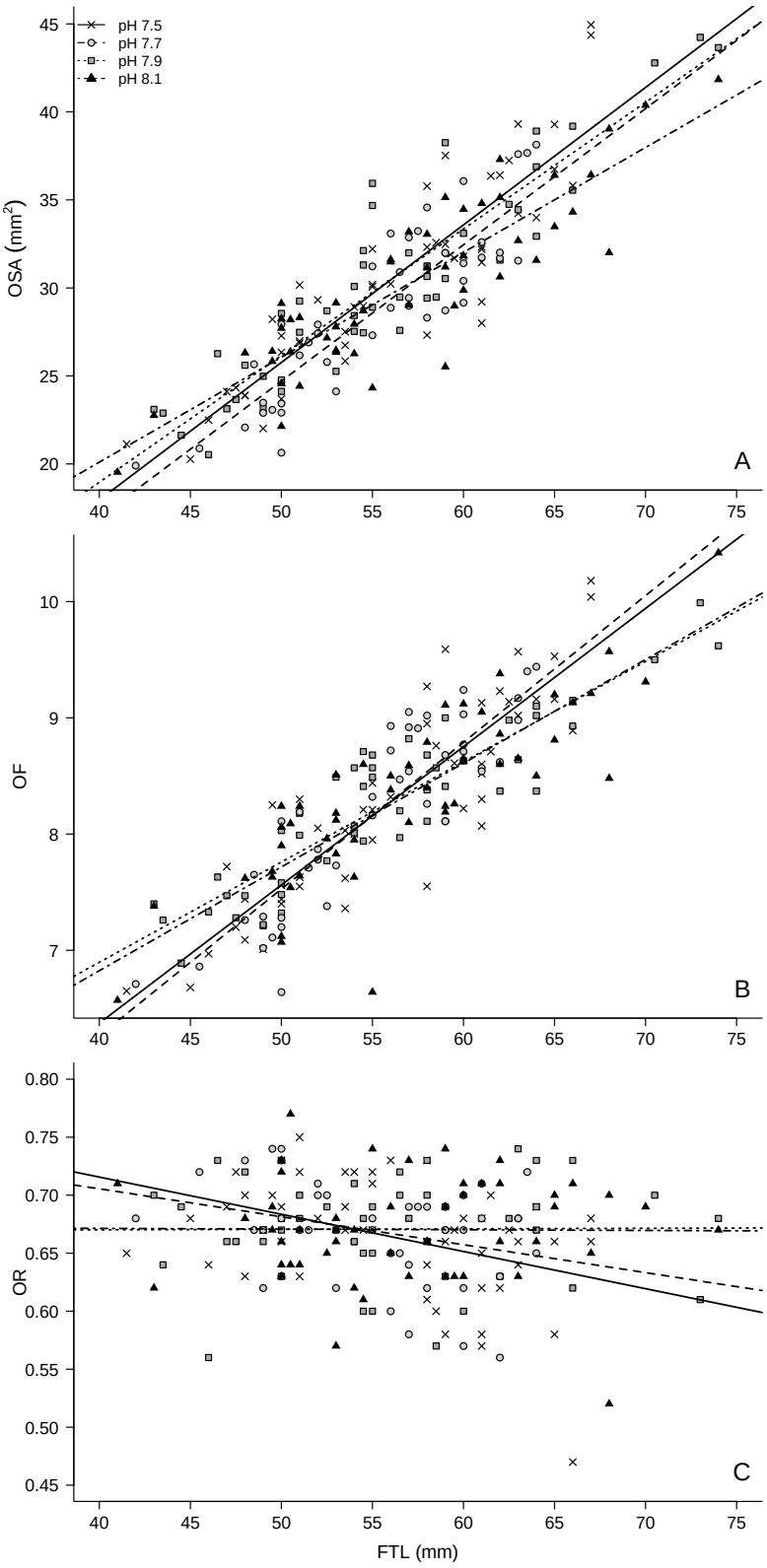
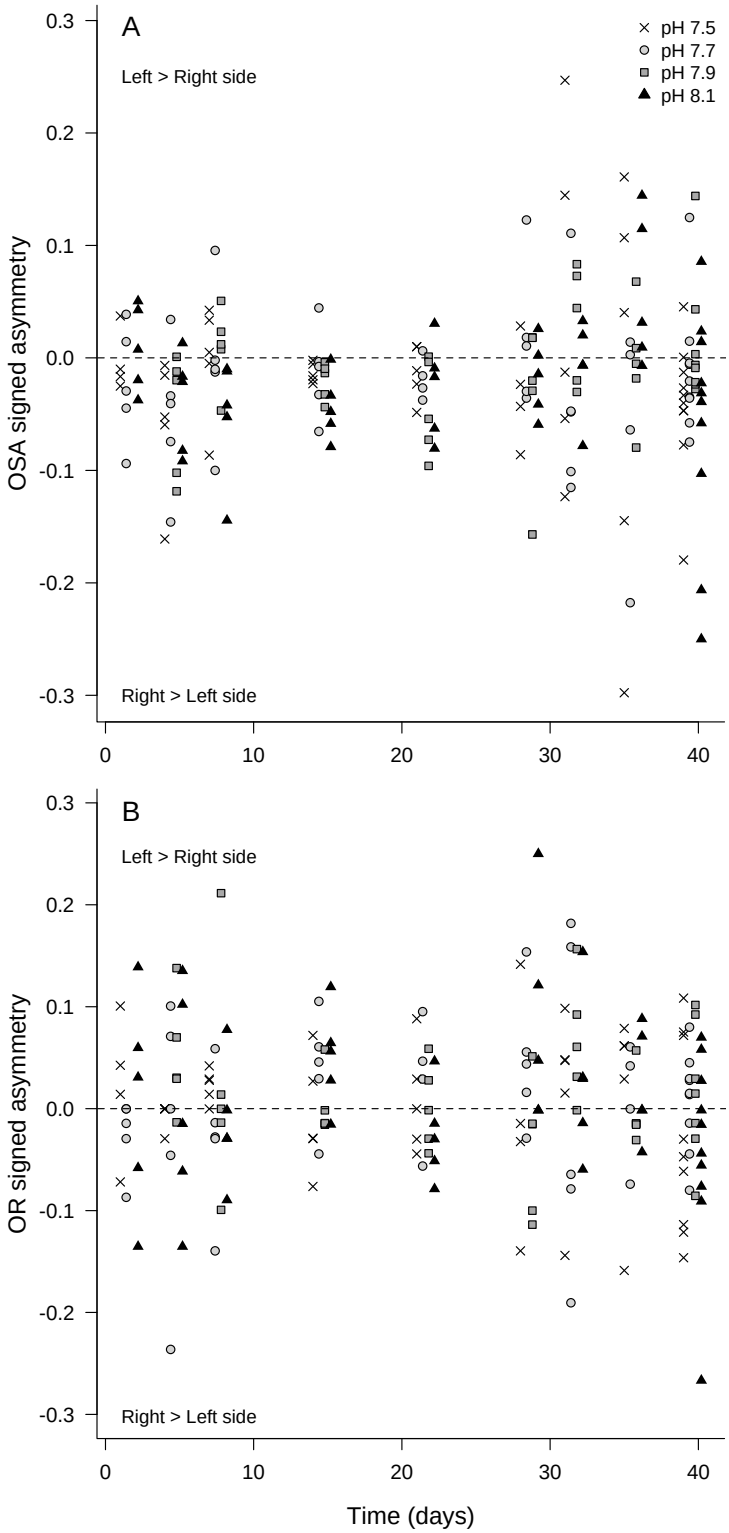


Figure 3. *Sparus aurata*. (**A**) Allometric relationship between the otolith surface area (OSA, mm^2) and the fish total length (FTL, mm), (**B**) Otolith Feret maximum diameter (OF) and (**C**) otolith roundness (OR) measured in juvenile seabreams raised during 40 d at four different pH_T treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment, see Table 2 for statistical significance). An otolith roundness value of 1 means a circular shape. For OR, only data recorded at pH 7.5 were significantly linearly distributed (full black line, p = 0.0046).



586

587 **Figure 4**. *Sparus aurata*. Asymmetry ([left - right]/mean$_{left,right}$) in (**A**) otolith surface area (OSA)
588 and (**B**) otolith roundness (OR) between left and right sagitta of juveniles reared at 4 pH
589 treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment) during 40 d. Dashed-line represents
590 symmetry; offset in the *x*-axis was used to display the data per pH treatment.

591

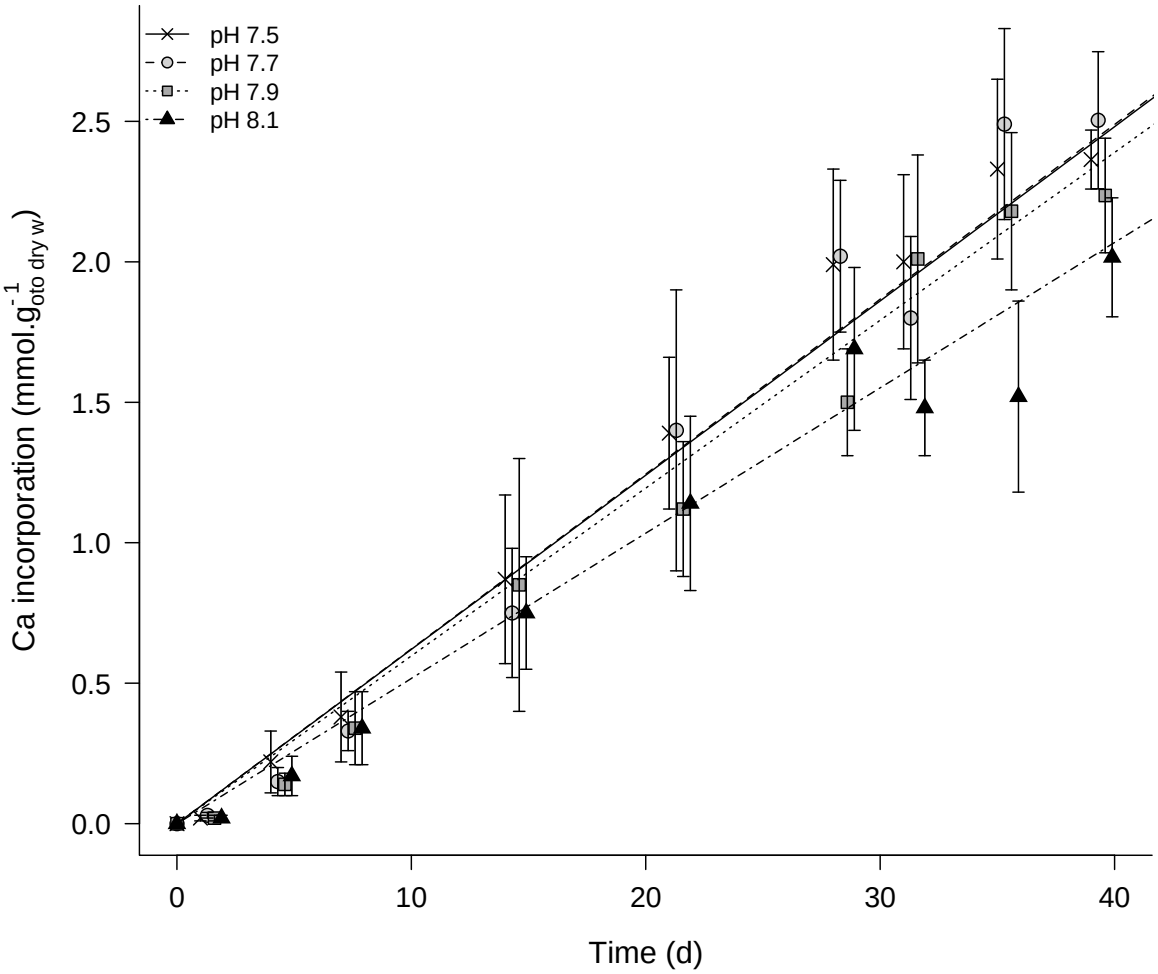


Figure 5. *Sparus aurata*. Incorporation of calcium (mmol.$g_{\text{otolith dry weight}}^{-1}$ ± sd) in sagittal otolith of juvenile seabreams reared at 4 pH treatments 7.5, 7.7, 7.9, and 8.1 (1 tank per treatment) during 40 d. Offset in the *x*-axis was used to display the data per pH treatment. See Table 3 for data and statistical comparisons. Rates of Ca incorporation calculated on pseudoreplicates at pH_T 8.1 are 18, 27 and 25% lower than rates at pH_T 7.9, 7.7 and 7.5, respectively.